

IN VITRO CYTOSTATIC ACTIVITY OF 8-SUBSTITUTED AND TRICYCLIC ANALOGUES OF ACYCLOVIR

Bogusław Hładoń[#], Tomasz Gośliński**, Halina Laskowska*, Daniel Baranowski**, Tomasz Ostrowski**, Joanna Zeidler**, Piotr Ruszkowski*, Bożenna Golankiewicz***

* Department of Pharmacology, Karol Marcinkowski University of Medical Sciences, Fredry 10, PL 61-701 Poznań, Poland, **Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, PL 61-704 Poznań, Poland

In vitro cytostatic activity of 8-substituted and tricyclic analogues of acyclovir: B. HŁADOŃ, T. GOŚLIŃSKI, H. LASKOWSKA, D. BARANOWSKI, T. OSTROWSKI, J. ZEIDLER, P. RUSZKOWSKI, B. GOLANKIEWICZ. *Pol. J. Pharmacol.*, 2002, 54, 45–53.

Out of a series of twenty 8-substituted or/and 1,N-2-bridged (tricyclic) derivatives of acyclovir (a selective antiherpetic drug), known to be nontoxic to normal cells, seven compounds were found to exhibit moderate cytostatic activity in KB human tumor tissue culture system with ED₅₀ activity values ranging from 0.052–0.094 × 10⁻³ mole/l.

The structure-activity relationship analysis indicated that the primary factors determining their cytotoxicity were: 1) bromine atom at the C-8 position of the bicyclic derivatives and 2) unsubstituted appended ring in the tricyclic derivatives. Combination of two structural elements carrying the cytotoxicity gave diverse effects, enhancement or decrease in activity depending on particular cases.

Two compounds (of four selected), 8-bromoacyclovir and 1,N-2-ethenoacyclovir, having unsubstituted 9-[(2-hydroxyethoxy)methyl] chain, showed approximately 2-fold increase in their cytotoxicity against HeLa tumor cells in the presence of the induced microsomal generating system suggesting that their cytotoxicity depends on the drug metabolic transformation into their active metabolites (intermediates) *via* MFO-system, and that structural unit of this chain is essential for abovementioned activation.

Presently found remarkable cytotoxic selectivity of acyclovir analogues against KB and HeLa tumor cells together with previously reported in the literature specific cytotoxic activity of acyclovir against murine leukemia L 1210 cells seem to be encouraging for further investigation of this class of compounds in other tumor systems.

Key words: *acyclovir analogues, cytotoxic activity*

INTRODUCTION

Acyclovir, 9-[(2-hydroxyethoxy)methyl]guanine (ACV) is a well known, highly potent and selective antiherpetic drug, capable of inhibiting the replication of both HSV-1 and HSV-2 herpes simplex viruses at levels far below the cytotoxic concentrations [6]. It is known that this acyclic analogue of guanosine is recognized as a substrate by the virus-specific dThd kinase [7]. The lack of such recognition by host cell enzymes results in a low level of toxicity to uninfected cells.

Although ACV is considered to be essentially an antiviral agent, it has been found to have the growth inhibitory activity against murine leukemia L 1210 cells at a relatively low concentration of 13 µg/ml [19]. This activity has turned out to be very specific for ACV itself as out of nine of its 8 substituted derivatives tested, only 8-iodo analogue inhibited proliferation of L 1210 cells at concentrations below 300 µg/ml [19]. Therefore, the investigation of further derivatives might prove useful to explore other pharmacological directions.

As we were interested in the examination of the selective cytostatic activity against KB and HeLa human cancer cells, we chose two classes of ACV analogues known as nontoxic to normal cells: abovementioned 8-substituted and tricyclic. The latter have been prepared previously by linking together 1 and N-2 positions of guanine moiety of ACV with substituted ethene-1,2-diyl bridge to form various 3,9-dihydro-9-oxo-5*H*-imidazo[1,2-*a*]-purine (TACV)* derivatives [1, 2, 9,10].

We report now the data on the cytotoxicity of twenty ACV analogues and the relation of activity of the most potent of them to the metabolic transformation *via* mixed function oxidases (MFO) – cytochrome P-450 system.

MATERIALS and METHODS

Compounds

Twenty analogues of ACV: four 8-substituted and sixteen tricyclic, 3,9-dihydro-9-oxo-5*H*-imidazo[1,2-*a*]purine derivatives – “T” congeners were studied.

The compounds, synthesized at the Institute of Bioorganic Chemistry, Polish Academy of Sciences,

Poznań, Poland (IBC, PAS) had defined physicochemical parameters and established chemical structure. ACV (IBC, PAS) was a reference standard and cytosine arabinoside (Upjohn, Belgium) and actinomycin D (Merck, Sharp and Dohme, USA) were used as the secondary standards. The chemical structures of the tested compounds are given in Figure 1.

Special solubility

The compounds were dissolved under optimal conditions (at 20°C), at the maximal concentration of 2 mg/cm³ in water with addition of 0.0033% DMSO (Merck, Germany). Final solvent concentration was adjusted so that it did not exceed a predetermined nontoxic level (which did not inhibit the control cell proliferation or protein biosynthesis).

In vitro studies were based on the programs of Drug Research and Development, Division of Cancer Treatment DR and D, National Cancer Institute (NCI), National Institute of Health (NIH), Bethesda, MD, USA with necessary modifications [5, 8, 11–13, 16]. The studies were carried out on tissue culture of human KB (nasopharynx carcinoma) and HeLa 3S (carcinoma cervicis uteri) tumor cell lines (PZH, Warszawa, Poland).

Tissue culture

Stock cultures were maintained by the periodic (4 to 6 days) transfer method in the Eagle (MME) and Parker (P199) cultures media supplemented with 10% calf serum; in KB (MME₉₀ serum₁₀) and HeLa (P199₁₀ serum₁₀) cell systems, respectively [16]. Sera and tissue culture media were obtained from the Serum and Vaccine Production Laboratories in Lublin. For experiments, cells in the logarithmic phase of growth under culture condition (time of compound exposure : 72 h (for KB system), pH value of the medium : 7.2) were harvested by trypsinization and centrifuged prior to investigation [16].

Cytotoxic activity testing

The procedure of Eagle and Foley [5] modified by Smith et al. [20] was used. Cytotoxic activity was determined by the ability to inhibit the biosynthesis of cellular protein. Levels of cellular protein were determined by the method of Oyama and

* The tricyclic modified guanine moiety, the systematic name of which is 3,9-dihydro-9-oxo-5*H*-imidazo[1,2-*a*]purine is designated throughout this paper by adding “T” to the abbreviation of the name of the parent compound.

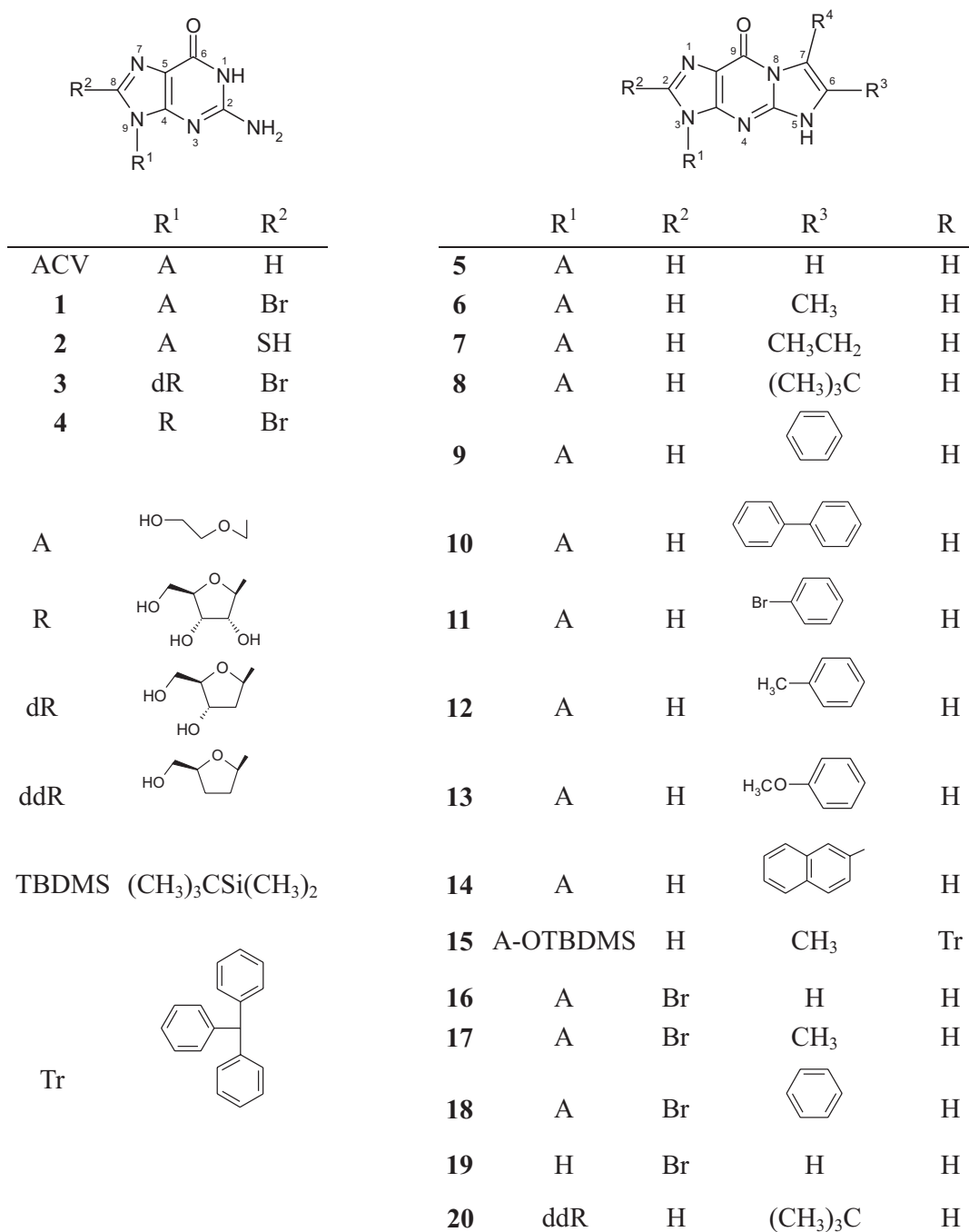


Fig. 1. Structural formulas of 8-substituted and tricyclic analogues of acyclovir (ACV)

Eagle [17] using crystalline bovine albumin as a standard. ED₅₀ represents the dose which inhibits the increase in cellular protein by 50% as compared with the control cultures. The technical details of the procedures have been given elsewhere [8, 12, 13, 16].

Microsomal preparations

Male Wistar albino rats (inbred colony) weighing 200–250 g were used for preparation of liver microsomes. The livers were excised under sterile conditions, cooled, and homogenized in 4 volumes of PBS, phosphate buffered saline, pH 7.4 at 0°C

with a Potter-Elvehjen homogenizer; 9000 × g supernatant was prepared by centrifugation of 25% liver homogenates in 0.1 M Tris-HCl buffer, pH 7.4 for 20 min. The supernatant was removed and centrifuged in a refrigerated Beckman-L-20 ultracentrifuge at 105000 × g for 40 min. The microsomal pellet was washed by resuspending in PBS with teflon pestle and recentrifuged at 105000 × g for 1 h. The volume of the final microsomal suspension was adjusted at 0–4°C to contain microsomes from 1 g of liver wet weight per ml of PBS.

Tissue culture in the presence of generating systems with cofactors

HeLa cells were grown as monolayer in roller tubes in Parker medium supplemented with 10% calf serum at the concentration of 10⁵ cells per 1 ml of medium. The growth medium in the tubes was changed as described previously. After 48 h incubation at 37°C the following constituents were given to each test tube: 1 ml of PBS containing microsomes equivalent to 250 mg of liver; 1 ml of PBS containing compounds corresponding to concentration range between 1–1000 µg/cm³ with 5 levels of concentration at 0.3 log intervals, and 1 ml of PBS containing the following cofactors: 10 mg of glucose-6-phosphate (disodium salt), 1.2 mg of NADP (sodium salt), 2.1 mg of MgCl₂ × 6 H₂O, 5.0 µl of glucose-6-phosphate dehydrogenase (0.5 units) in a total volume of 3 ml. The test tubes were covered with aluminium caps, gently mixed and incubated at 37°C, for 1 h. After incubation, the mixture was removed with sterile syringe, test tubes were gently rinsed with PBS, and to each tube 4 ml of P199 medium containing antibiotics (100 units of penicillin and 50 µg of streptomycin/ml) with 10% calf serum was added and incubated again at 37°C for 24 h, as compared with control non-induced samples, i.e. without the microsomes (M = 250 M) and cofactors (CF) constituents (see the explanations in Table 3).

Evaluation of the results

The two-grade system of evaluation and calculation of results proposed by NCI, NIH was used. Compounds were qualified for the second step, if their activity measured at 3 concentrations (at 1 log intervals) against HeLa cells was at least 6 µg/cm³. In the second step of the investigation, the compounds were examined only with KB cells at 5 levels of concentration (at 0.3 log intervals). Com-

pounds for which the average of ED₅₀ of the first and second test was 4 µg/cm³ or less, were qualified for confirmation (further *in vivo* investigation), while the compounds with ED₅₀ activity ranging from 4 to 30 µg/cm³, arbitrarily assumed as moderately active, were qualified for confirmation in other *in vitro* systems.

Statistical calculations

The statistical significance of differences between the means of the samples was assessed by the Student's *t*-test for populations of different sizes. Final evaluation of the results was carried out by a multistage computer analysis system, using the CY-VTP/1 and CY-VTP/2 programs [13].

RESULTS

Some of the investigated compounds were poorly soluble in water. As this property could lead to incorrect interpretation of the results, special solubility assays were performed.

The solubility data are presented in Tables 1 and 2. As they depended on the technical conditions of the test, in all cases the chosen DMSO concentration was below the toxic level, i.e. not exerting any inhibitory effect when compared with the control sample. This allowed to divide the tested compounds into three groups according to the following solubility classification: soluble compounds (over 2 mg/cm³) – degree 3, moderately soluble (between 0.5–2 mg/cm³) – degree 2, and weakly or poorly soluble (below 0.5 mg/cm³) – degree 1.

On the basis of the obtained results, among 8-substituted analogues of ACV, two compounds, 8-Br-ACV (**1**) and 8-Br-dGuo (**3**), were found to be moderately active with their ED₅₀ (KB) of 0.064 × 10⁻³ and 0.094 × 10⁻³ mole/l, respectively. In addition, these compounds demonstrated good (degree 3) and moderate (degree 2) solubility (Tab. 1 and 2).

Compounds 8-SH-ACV (**2**) and 8-Br-Guo (**4**) showed low activity under the above conditions, with ED₅₀ over the 0.1 × 10⁻³ mole/l.

In the group of tricyclic (TACV) derivatives, the most active compounds appeared to be: 6-Me-7-Tr-OTBDMS-TACV (**15**), 2-Br-6-Ph-TACV (**18**), 2-Br-TACV (**16**), TACV (**5**) and 2-Br-TGua (**19**) with ED₅₀ (KB) ranging from 0.052 to 0.095 × 10⁻³ mole/l and with diverse solubility (Tab. 1 and 2).

Other C-6 substituted TACV analogues were classified as having low activity (compounds **6**, **9**,

Table 1. Cytotoxic activity of acyclovir (ACV) analogues against KB human tumor cells

No.	Compound Int. code	MW	Solubility ^{a)} at 20°C H ₂ O + DMSO	ED ₅₀ ^{b)}			R ^{d)}	Remarks ^{e)}
				µg/cm ³ (mole/l × 10 ⁻³)	Range	ED ₀ ^{c)}		
8-Substituted analogues								
1	8-Br-ACV	304.11	2	19.5 (0.0641)*	13–31	3.2	80	MA
2	8-SH-ACV	257.27	2	45.0 (0.1749)*	28–74	6.5	85	LA
3	8-Br-dGuo	267.24	3	25.0 (0.0935)*	15–40	3.9	85	MA
4	8-Br-Guo	283.24	3	38.0 (0.1341)*	22–62	5.8	80	LA
Tricyclic analogues								
5	TACV	249.23	2	23.0 (0.0922)*	11–36	2.4	79	MA
6	6-Me-TACV	263.26	2	37.0 (0.1405)*	22–60	5.1	80	LA
7	6-Et-TACV	277.28	2	58.0 (0.2092)	36–95	7.0	90	NA
8	6-t-Bu-TACV	305.34	2	> 500 (> 1.6375)	–	–	60	NA
9	6-Ph-TACV	325.30	2	42.0 (0.1291)*	26–69	5.8	80	LA
10	6-Ph Ph-TACV	401.42	2	98.0 (0.2441)	80–120	15	69	NA
11	6-(4-Br Ph)-TACV	404.22	1	200 (0.4947)	90–150	35	65	NA
12	6-(4-Me Ph)-TACV	339.35	2	> 500 (> 1.4734)	–	–	60	NA
13	6-(4-MeO Ph)-TACV	355.35	2	> 500 (> 1.407)	–	–	60	NA
14	6-(2-Napht)-TACV	375.39	1	49.0 (0.1305)*	29–80	6.8	90	LA
15	6-Me-7-Tr-OTBDMS-TACV	619.80	1	32.0 (0.0516)*	20–54	5.0	78	MA
16	2-Br-TACV	328.13	3	26.0 (0.07924)*	16–43	3.6	90	MA
17	2-Br-6-Me-TACV	342.15	2	200 (0.5845)	80–120	–	65	NA
18	2-Br-6-Ph-TACV	404.22	3	28.0 (0.6927)*	17–45	3.8	65	MA
19	2-Br-T Gua	254.05	1	24.0 (0.09447)*	14.5–39	3.5	90	MA
20	6-t-Bu-dd T Guo	331.34	2	35.0 (0.1056)*	21–56	5.6	80	LA
	Reference compound ACV	225.21	2	100.0 (0.444)*	80–150	17.0	80	NA
	Standards:	–	–					
	Cytosine arabinoside (Upjohn)			0.93–1.04*	–	–	–	–
	Actinomycin D			0.06*				
	(Merck Sharp and Dohme)	–	–		–	–	–	–

^{a)} Degree of special solubility: the soluble compounds (over 2 mg/cm³ – degree 3), moderately (middle) soluble (between 0.5–2 mg/cm³ – degree 2), weakly or poorly soluble (below of 0.5 mg/cm³ – degree 1), in a solvent mixture containing 0.0033% DMSO in water. ^{b)} Concentration of a compound inhibiting by 50% protein biosynthesis in the cell population under culture conditions, time of exposure: 72 h, pH value of the medium: 7.2. ED₅₀ was calculated by computer analysis. The compounds were tested at 5 levels of concentration with 0.3 log intervals. Standard deviations of log ED₅₀ did not exceed 0.26. The mean value of the compound activity at 95% probability was within the activity limits of ED₅₀ = y, from y/3.4 to y × 3.4. ^{c)} Minimum effective dose. ^{d)} The linear correlation coefficient. ^{e)} Moderately active compound MA, low active LA, inactive NA. *) p < 0.05 as compared to control (not exposed to the compounds)

14, 20 having ED_{50} over 0.1, ranging from 0.10 to 0.14×10^{-3} mole/l) or inactive (**7, 8, 10–13, 17**; ED_{50} over 0.2×10^{-3} mole/l) (Tab. 1 and 2). ACV, a reference compound, did not display cytotoxic activity ($ED_{50} = 0.444 \times 10^{-3}$ mole/l).

Two standards, cytosine arabinoside and actinomycin D ($ED_{50} = 0.93$ and $0.06 \mu\text{g}/\text{cm}^3$, respectively), showed activity comparable with NCI, NIH screening data protocols, confirming the correctness and sensitivity of our model study.

Four moderately active compounds **1, 3, 5** and **15**, two from each group of analogues, were qualified for further investigation in the rat liver microsomal generating *in vitro* system. Two compounds: 8-Br-ACV (**1**) and TACV (**5**) showed a considerable, approximately 2-fold, increase in their activity (Tab. 3).

In the group of 8-bromo substituted ACV analogues, cytotoxic activity of 8-Br-ACV (**1**) was nearly 2-times higher ($ED_{50} = 0.035 \times 10^{-3}$ mole/l) as compared to microsomally non-induced samples ($ED_{50} = 0.064 \times 10^{-3}$ mole/l), in contrast to 8-Br-dGuo (**3**) which did not demonstrate enhancement of its activity in the induced conditions (the same ED_{50} of

0.094×10^{-3} mole/l, in both the induced and non-induced systems) (Tab. 3).

Likewise in the tricyclic class, TACV (**5**) ($ED_{50} = 0.046 \times 10^{-3}$ mole/l) exhibited 2-fold higher cytotoxic activity in the induced vs non-induced samples ($ED_{50} = 0.092 \times 10^{-3}$ mole/l), while its analogue 6-Me-7-Tr-OTBDMS-TACV (**15**) demonstrated no increase in the activity with identical ED_{50} value of 0.084×10^{-3} mole/l, when both systems mentioned above were compared (Tab. 3).

As it was expected, actinomycin D, used as a standard, did not show enhancement of the activity due to its known and quite different mechanism of action.

Considering the results obtained *in vitro* in the presence of generating systems, the rise in the cytotoxic effect of 8-Br-ACV and TACV can be expected after *in vivo* phenobarbital premedication (the latter known as good inducer of metabolic transformation *via* MFO-cytochrome P-450 system), which is likely to be connected with the acceleration of the rate of drug metabolism, i.e. faster transformation into cytotoxically active metabolites or intermediates.

Table 2. 8-Substituted and tricyclic analogues of acyclovir (ACV) classified according to cytotoxicity

Cytotoxicity category ^{e)}	8-substituted analogues	$ED_{50}^b)$ mole/l $\times 10^{-3}$	Tricyclic analogues	$ED_{50}^b)$ mole/l $\times 10^{-3}$
MA	1 8-Br-ACV	0.0641 (2)	15 6-Me-7-Tr-OTBDMS	0.0516 (1)
	3 8-Br-dGuo	0.0935 (3)	18 2-Br-6-Ph-TACV	0.0693 (3)
			16 2-Br-TACV	0.0729 (3)
			5 TACV	0.0922 (2)
			19 2-Br-TGua	0.0945 (1)
LA	4 8-Br-Guo	0.1341 (3)	20 6-t-Bu-dd Guo	0.1056 (2)
	2 8-SH-ACV	0.1749 (2)	9 6-Ph-TACV	0.1291 (2)
			14 6-(2-Napht)-TACV	0.1305 (1)
			6 6-Me-TACV	0.1405 (2)
NA	Reference standard ACV	0.444 (2)	7 6-Et-TACV	0.2092 (2)
			10 6-Ph Ph-TACV	0.2441 (2)
			11 6-(4-Br Ph)-TACV	0.4947 (1)
			17 2-Br-6-Me-TACV	0.5845 (2)
			13 6-(4-MeO Ph)-TACV	1.4070 (2)
			12 6-(4-Me Ph)-TACV	1.4734 (2)
			8 6-t-Bu-TACV	1.6375 (2)

^{a,b,e)} See the explanations in Table 1. Degree of solubility ^{a)} is given in parentheses

Table 3. Comparison of the cytotoxic activity of acyclovir (ACV) analogues against tumor cells in the presence and absence of microsomal generating system

Compounds and/or generating system	ED ₅₀ ^{a)} (HeLa)			R ^{d)}
	μg/cm ³ ^{b)} (mole/l × 10 ⁻³)	range μg/cm ³	ED ₀ μg/cm ³ ^{c)}	
M (250M) ^{e)}	NA	–	–	–
CF ^{f)}	NA	–	–	–
M (250M) + CF	NA	–	–	–
8-Br-ACV (1)	19.5 (0.064)	13–31	3.2	85
8-Br-ACV + 250 M + CF	10.8 (0.035)*	2.8–18.0	1.8	95
8-Br-d Guo (3)	25.0 (0.094)	15–40	3.9	85
8-Br-d Guo + 250 M + CF	25.0 (0.094)	15–40	3.9	82
TACV (5)	23.0 (0.092)	11–36	2.4	79
TACV + 250 M + CF	11.5 (0.046)*	1.7–18.0	1.4	80
6-Me-7-Tr-OTBDMS-TACV (15)	52 (0.084)	33–71	8.0	75
6-Me-7-Tr-OTBDMS-TACV + 250 M + CF	52 (0.084)	33–71	8.0	75
Actinomycin D	0.06	(0.04–0.10)	0.01	85
Actinomycin D + 250 M + CF	0.05	(0.03–0.09)	0.01	82

^{a)} Concentration of a compound inhibiting by 50% protein biosynthesis in the cell population. ^{b,c,d)} See the explanations in Table 1. ^{e)} M – Microsomes (105000 × g liver fraction) equivalent to 250 mg of wet liver (M). ^{f)} CF = 10 mg of glucose-6-phosphate (disodium salt), 1.2 mg of NADP (sodium salt), 2.1 mg of MgCl₂ × 6H₂O, 5.0 μl of glucose-6-phosphate dehydrogenase (0.5 units). The time of incubation after the induction at 37°C was 1 h (see Methods). NA – non active. *) p < 0.05 as compared to non-induced (250 M + CF) controls

DISCUSSION

No marked solubility-activity relationship was observed.

The structure-activity relationship (SAR) analysis of the 8-substituted ACV analogues showed that the most important structural element for their cytotoxic effect against KB human tumor cells was the presence of the 8-bromo substituent. Replacement of a bromine atom in 8-Br-ACV (**1**) (ED₅₀ 0.064 × 10⁻³ mole/l) by a mercapto group resulted in 8-SH-ACV (**2**) exhibiting low activity. Smaller decrease in the activity followed the exchange of the 9-[(2-hydroxyethoxy)methyl] side chain into a deoxyribosyl or ribosyl moiety. 8-Br-dGuo (**3**) remained moderately active, while its ribosyl counterpart 8-Br-Guo (**4**) fell to the low activity category but was more active than **2**.

The tested TACV congeners consisted mainly of various 6-substituted derivatives (**6–15**). Generally any substitution at this position resulted either in the decrease (6-Me-TACV, **6**, 6-Ph-TACV, **9** and 6-Napht-TACV, **14**) or complete loss of activity (compounds **7**, **8**, **10–13**). The exceptional activity of 6-Me-7-Tr-OTBDMS-TACV (**15**), the most active of the whole series (ED₅₀ 0.052 × 10⁻³ mole/l) may be due to its 7-substituent. It has been found recently that 7-substitution of TACV with trityl group gives rise to enhancement of toxicity against normal cells [10].

The effect of combination of two structural elements carrying the cytotoxicity against KB human tumor cells was diverse: (1) about twofold (6-Ph-TACV/2-Br-6-Ph-TACV, 0.13/0.069 mole/l × 10⁻³) or slight (TACV/2-Br-TACV, 0.092/0.079) enhancement and, on the contrary, (2) marked decrease (6-Me-TACV/2-Br-6-Me-TACV, 0.14/0.58). It is

noteworthy that the resultant compounds 2-Br-TACV and 2-Br-6-Ph-TACV were better soluble (degree 3) than their parent compounds 8-Br-ACV and TACV.

The influence of the kind of sugar or pseudo-sugar moiety on the cytotoxicity level was also diversified. 2-Br-TGua **19**, devoid of that part of molecule, was still moderately active, and its activity was comparable with that of 2-Br-TACV. The presence of dideoxyribosyl moiety in 6-t-BuddTGua, **20**, caused recovery of the activity to the level close to that of TACV (**5**) and made **20** 15-fold more active than its acyclo congener 6-t-Bu-TACV (**8**).

However, the results of the experiments performed with rat liver microsomal preparations on four most active compounds showed that only **1** and **5**, having unsubstituted 9-[(2-hydroxyethoxy)-methyl] chain exhibited considerable, approximately 2-fold increase in their *in vitro* cytotoxicity in contrast to compounds **3** and **15**, the cytotoxicity of which did not change. This observation suggests that in the system simulating *in vivo* drug metabolic activation the abovementioned chain structural unit is essential.

The inhibitory activity of TACV seems to be specific for the KB cells because this tricyclic analogue has been previously found nontoxic to HEL (human embryonic lung) cells at a concentration of 200 $\mu\text{g}/\text{cm}^3$ [2].

Thus, according to the criterion accepted for the selection of antitumor agents with growth inhibitory capacity determined on the basis of their *in vitro* activity (C microsomal generating system), two compounds 8-Br-ACV (**1**) and TACV (**5**), out of twenty investigated, were qualified for the subsequent studies in the transplantable animal tumor model systems.

The tricyclic analogues of ACV are considered to be intrinsically active as antiviral agents on the basis of their selectivity of action [1] and complex formation with herpesvirus type 1 deoxythymidine kinase [4]. On the other hand, it has been previously found that guanine moiety can be regenerated chemically from the tricyclic analogues by some oxidative agents [3]. One could assume that tricyclic analogues are ACV prodrugs which might be cleaved to parent compound by metabolic transformation in the liver. Presently observed enhancement of cytotoxicity of TACV in rat liver microsomal generating system provides evidence against such doubts.

In conclusion, we have found remarkable cytotoxic selectivity of some acyclovir analogues against

KB and HeLa tumor cells which together with previously reported in the literature specific cytotoxic activity of ACV against murine leukemia L 1210 [19] seems to be encouraging for further investigation of this class of compounds in other tumor systems.

Experimental. Chemistry

The majority of the compounds tested were described previously and prepared according to the following literature references: **1** [19]; **2** [18]; **3** [15]; **4** [14]; **5** [2]; **6** [1]; **7**, **12–14** [10]; **8–11**, **17** [9]; **15** [22]. For new compounds **16**, and **18–20** general methods were as given in [9]; in the particular, appropriately modified substrates were prepared according to procedures described for analogous compounds: **16,19** [2], **18** [9], **20** [21].

2-Bromo-3,9-dihydro-3-[(2-hydroxyethoxy)-methyl]-9-oxo-5H-imidazo [1,2-a] purine (16) and **2-bromo-1(3),9-dihydro-9-oxo-5H-imidazo [1,2-a] purine (19)**.

When intermediate acetal derivative of **1** reacted with 40% acetic acid in bath heated at 145°C, the main product **16** was partly hydrolyzed to **19**. The mixture was separated on a chromatographic short column (Merck TLC silica gel 60H) in $\text{CDCl}_3/\text{MeOH}$; 92.5:7.5 (v/v) to isolate **16** and **19** in quantitative molar proportion 3:2. **16**: Colorless small crystals (EtOH). Mp > 201°C dec. ^1H NMR (DMSO-d_6) 2.56, 3.48 (2xt, CH_2CH_2); 4.69 (t, 1, OH); 5.46 (s, 2, N- CH_2 -O); 7.49 (d, 1, H-6); 7.66 (d, 1, H-7); 12.58 (brs, 1, N-5-H) ^{13}C NMR (DMSO-d_6): 59.79 (Tt, CH_2); 70.92 (Tt, CH_2); 72.64 (Tt, N- CH_2 -O); 107.21 (Dd, C-6); 115.10 (S, C-9a); 116.60 (Dd, C-7); 122.57 (S, C-2); 145.75 (Sm, C-4a); 150.06 (S, C-3a); 151.64 (S, C-9a) Analysis for $\text{C}_{10}\text{H}_{10}\text{BrN}_5\text{O}_3$ (328.13) C, H, N **19**: Colorless small crystals ^1H NMR (DMSO-d_6) 7.45 (d, 1, H, H-6); 7.61 (d, 1, H-7); 12.42 (brs, 1, N-5-H); 13.33, 13.85 (2 \times brs, N-3(1)-H)

2-Bromo-3,9-dihydro-3-[(2-hydroxyethoxy)-methyl]-9-oxo-6-phenyl-5H-imidazo [1,2-a] purine (18). Colorless crystals (EtOAc/MeOH; 10:7) mp > 200°C dec.: ^1H NMR (DMSO-d_6) 3.51–3.59 (m, 4, CH_2CH_2); 4.72 (t, 1, 5'-OH); 5.48 (s, 2, N- CH_2 -O); 7.49, 7.92 (d, 2, m, 3, 6-Ph); 8.25 (s, 1, H-7); 13.28 (brs, 1, N-5-H) ^{13}C NMR (DMSO-d_6) 59.85 (Tm, CH_2); 70.93 (Tm, CH_2CH_2); 72.77 (Tm, N- CH_2 -O); 103.51 (C-7); 115.46 (C-9a); 122.61 (C-2); 6-Ph (125.01, C1'; 127.68 C-ortho; 128.78 C-meta; 128.96 C-para); 129.24 (C-6); 148.28 (C-4a); 150.00 (C-3a); 151.48 (C-9). Analysis for $\text{C}_{16}\text{H}_{14}\text{BrN}_5\text{O}_3$ (404.22) C, H, N.

6-t-Butyl-3-(2,3-dideoxy- β -D-ribofuranosyl)-3,9-dihydro-9-oxo-5H-imidazo[1,2-a]purine (20). Colorless crystals (EtOAc/MeOH; 5:1) mp 235°C dec. ^1H NMR (DMSO- d_6): 1.32 (s, 9H, *t*-Bu), 2.02 (m, 2H, 3', 3''-H); 2.38 (m, 2H, 2', 2''-H); 3.52 (m, 1H, 5''-H); 3.65 (m, 1H, 5'-H), 7.25 (s, 1H, H-7), 8.16 (s, 1H, H-2), 12.51 (brs, 1H, ex, N-5-H). Analysis for $\text{C}_{16}\text{H}_{21}\text{N}_5\text{O}_3$ (331.39): C, H, N.

Acknowledgment. This work was partly supported by the State Committee for Scientific Research grants no. 4 PO5F 030 08 and 4 PO5F 005 16, Warszawa, Poland.

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Received: November 26, 2001; in revised form: January 14, 2002.